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A Novel Cyclotetrapeptide produced by *Lactobacillus helveticus* as a tyrosinase inhibitor

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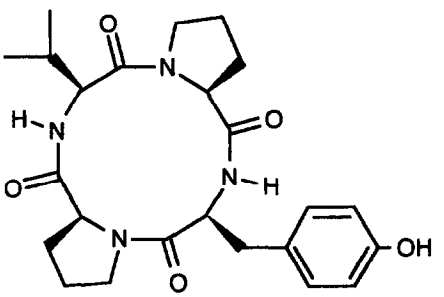
Abstract; A novel cyclotetrapeptide cyclo(-L-Pro-L-Tyr-L-Pro-L-Val-) was isolated from the lactic bacterium *Lactobacillus helveticus.* The structure was determined by spectroscopic means and confirmed by acid hydrolysis of the peptide and sequence analyses of the resulting linear peptides.

Tyrosinase (phenol oxidase) [EC 1.14.18.1] is known to be a key enzyme for melanin biosynthesis in plants, microorganisms and mammalian cells, and also known to be as a copper-containing enzyme[1-3]. Many compounds, such as kojic acid and arbutin, have been reported as tyrosinase inhibitors[4,5]. During our continuous screening of the inhibitors produced by lactic bacteria, we found the potent activity in *Lactobacillus helveticus.* This paper describes the isolation, structure, and activity of a novel tyrosinase inhibitor from the bacterium.



compound 1

Lactobacillus helveticus JCM 1120 was cultivated at 37°C for 18 hours under aerobic condition in Briggs medium. The cultured broth of the bacterium was filtered and the filtrate obtained (8 liter) was extracted with ethyl acetate. Chromatography of the extract

with Amberlite XAD-7 afforded several active fractions and silica gel column chromatography of the most active fraction followed by HPLC with an ODS column gave compound **1** (3.0 mg).

FABMS of **1** exhibited MH^+ ion at *m/z* 457. The NMR spectra of the compound were typical peptide-ones[6]. The amino acid analysis of **1** showed Tyr:Val:Pro = 1:1:2 (mole ratio), and all the amino acids had L-configuration on the basis of HPLC analyses with a chiral column. The sequence of the peptide was determined by HMBC in D_2O; cross peaks were observed between H1 of Tyr and C1 of one of two Pro (Pro1), and H1 of Pro1 and C1 of Val, indicating that **1** was cyclo(-L-Pro-L-Tyr-L-Pro-L-Val-). The sequence was confirmed by sequencer-analyses of the linear peptides obtained by hydrolysis of the cyclopeptide with 12M HCl at 37°C for 24 hours; two dipeptides, Pro-Tyr and Tyr-Pro, were detected in the analyses. Both the two peptides were not artifacts produced by the interchange between L-Pro-L-Tyr and L-Tyr-L-Pro via cyclo(L-Tyr-L-Pro-) since such a reaction did not occur under the same conditions by using synthetic two peptides.

This cyclopeptide showed strong inhibitory activity against mushroom tyrosinase; the concentration causing 50% inhibition (IC_{50}) was 1.5 mM. This activity was stronger than that of arbutin (5.0 mM) known as a potent inhibitor.

Many biologically active cyclopeptide are known, but only a few naturally occurring cyclotetrapeptides have been fully characterized; cyclo(-L-Pro-L-Leu-)$_2$, cyclo(-L-Pro-L-Val-)$_2$, and cyclo(-L-Pro-L-Phe-)$_2$ have been isolated from a marine ascidian as cytotoxic compounds toward L1210 cells[7].

References and Note

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6) ^1H NMR of **1** (GEOL GSX-400 spectrometer): Pro1; 0.74(m, H3a), 1.78(m H4), 1.92-2.07(m, H3b), 3.32(m, H5a), 3.47-3.61(m, H5b), 4.04(dd, J =11.54, 6.04, H2). Val; 0.86(d, J =7.60, Me), 1.07(d, J =7.32, Me), 2.47(m, H3), 4.16(br.s, H2). Pro2; 1.92-2.07(m, H4), 2.35(m, H3), 3.47-3.61(m, H5), 4.29(m, H2). Tyr; 3.00(dd, J =14.28, 4.03, H3a), 3.21(dd, J =14.28, 4.03, H3b), 4.53(br.s, H2), 6.86(d, J =7.69, Ph3,5), 7.07(d, J =7.69, Ph2,6). ^{13}C NMR of **1** (GEOL GSX-400 spectrometer): Pro1; 23.66(C4), 30.52(C3), 47.58(C5), 61.40(C2), 172.94(C1). Val; 17.95(Me), 20.81(Me), 31.77(C3), 63.21(C2), 169.98(C1). Pro2; 24.43(C4), 30.97(C3), 48.06(C5), 61.59(C2), 168.81(C1). Tyr; 40.23(C3), 59.40(C2), 118.21(Ph3,5), 129.56(Ph1), 134.29(Ph2,6), 157.64(Ph4), 168.81(C1). These assignments were established by the COSY, NOESY, DEPT, HMQC and HMBC experiments.
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